

Estimation of measurement uncertainty in food microbiology: *a normative approach*

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Outline

- The normative approach for food microbiology
- Measurement uncertainty in quantitative microbiology
- Measurement uncertainty in qualitative microbiology
- Interpretation against legal limits

Introduction

Why ?

- **Accreditation** of laboratories
 - Requirements of EN ISO 17025, § 5.4.6
 - To estimate the measurement uncertainty (MU) associated to the results produced by the laboratory
 - If rigorous/statistically valid calculation of MU not possible, to identify the MU components and to make a reasonable estimation of them
 - Implementation in France (COFRAC) for food microbiology
= delayed (see ISO works)

Why ? (foll.)

- **Significance** of microbiological analysis
= direct hazard for the consumer health
- **Quantitative** methods in microbiology
= highly **variable**
→ Need to quantify this variability
- How to express MU for **qualitative** determinations ?

The normative approach for food microbiology

The ISO structure

- ISO/TC 34/SC 9
 - TC 34 « Food products »
 - SC 9 « Microbiology »
- Microbiological analysis of foods & feeds
- « Horizontal » approach

Bangkok, December 2002

- MU in **quantitative** microbiology
= Basic approach adopted
 - Technical Specification (ISO/TS)
 - Quicker publication
 - Users' review & 2-year revision
- MU for **qualitative** determinations
= In a 2nd step

Measurement uncertainty in quantitative microbiology

The approach (1)

- Quantitative determinations
 - Colony-Count Techniques (+ Most Probable Number Techniques)
 - Alternatives methods (instrumental)
- Decision based on a 1st series of ISO trials (2002)
- Broad consensus at ISO meeting

The approach (2)

- GUM **decomposition** approach not selected
 - MU underestimation ?
 - Heavy in food microbiology

The approach (3)

- « Global » approach chosen
 - Reproducibility standard-deviation (s_R) on the final result of the entire measurement process
 - In agreement with
 - Codex Alimentarius (CCMAS)
 - ISO/TS 21 748 : 2004
 - « *Guide for use of the estimations of repeatability, reproducibility and trueness in the estimation of measurement uncertainty* »
 - Established by ISO/TC 69/SC 6
 - Bridge between GUM & ISO 5725
 - ↗ Global approach = special case of type A experimental estimation

The main steps

- 2003: 1st draft of ISO/TS 19036
 - « *Microbiology of foods and animal feeding stuffs – Guide for the expression of measurement uncertainty of quantitative determinations* »
- 2003/2004: 2nd series of ISO trials
 - To quantify MU component linked to
 - Sampling of the test portion
 - Preparation of the initial suspension
 - 78 participants, from 10 countries
- 2004: Decisions for final draft of ISO/TS 19036
 - ISO Project Group
 - ISO/TC 34/SC 9

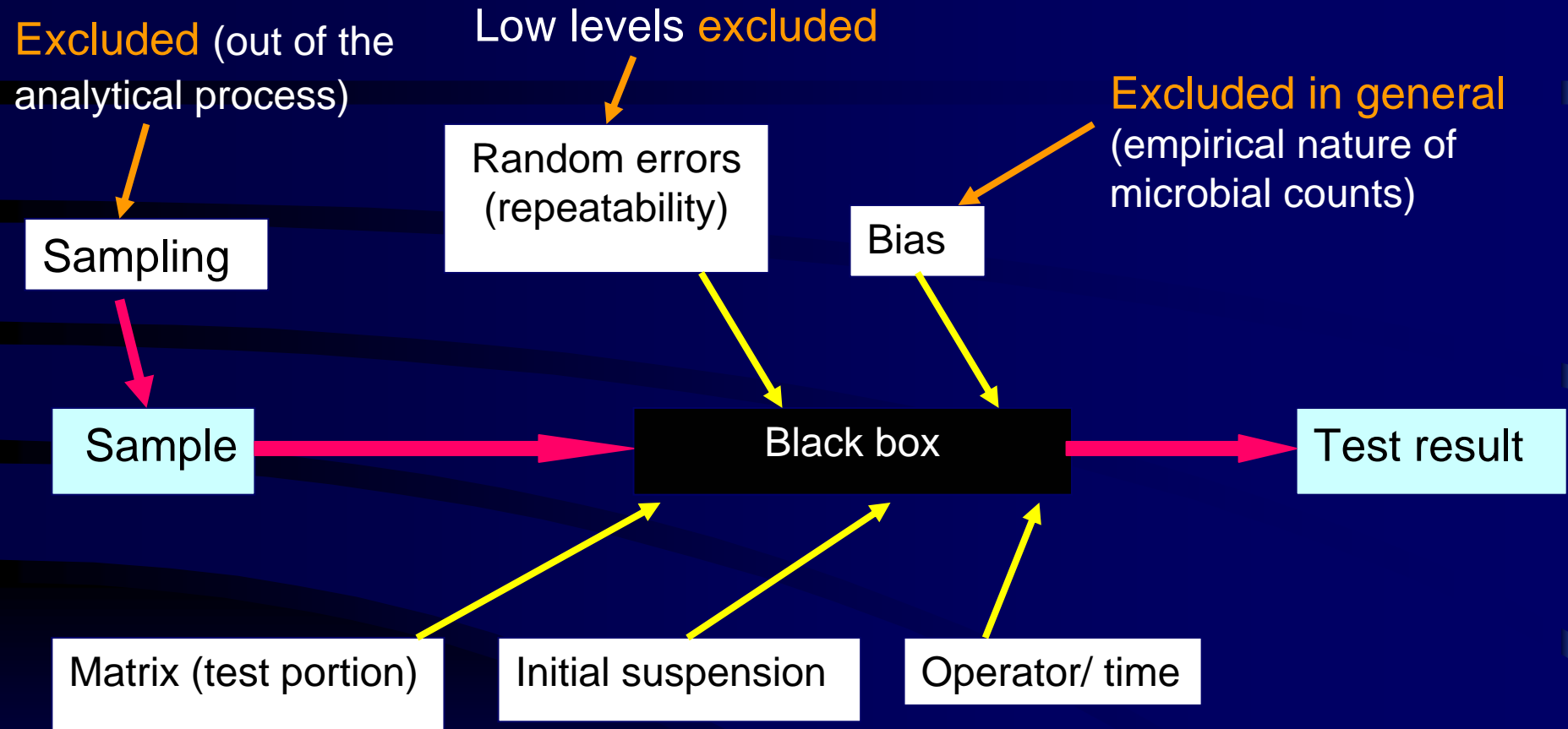
Guide ISO/TS 19036 - Presentation (1)

- Final Draft
 - ↳ Under 3-month vote (→ 11 May)
before publication
- Principles
 1. Global approach
 2. Enlarged MU = $2 s_R$

Guide ISO/TS 19036 - Presentation (2)

- Principles (foll.)
 3. s_R estimated per
 - (consistent group of) microorganism(s)
 - (consistent group of) matrix(ces)
 4. **3 options for s_R**
 - Intra-laboratory s_R
 - Inter-laboratory s_R (method validation)
 - Inter-laboratory s_R (proficiency testing)

The « black-box » diagram

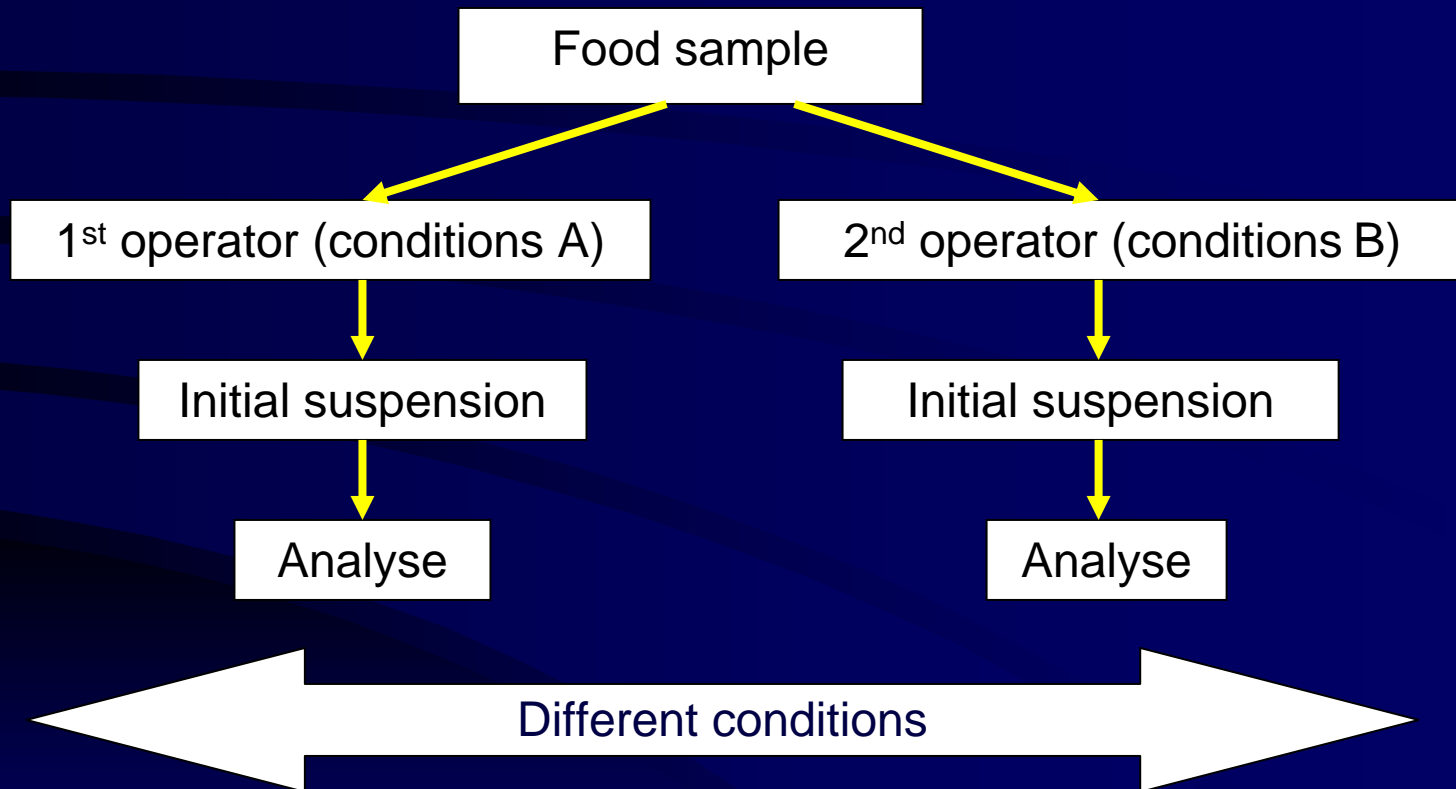


Intra-laboratory s_R (1)

- 1st (preferred) option
 - ↳ linked to the laboratory *per se*

Intra-laboratory s_R (2)

Experimental protocol



Intra-laboratory s_R (3)

- The experimental protocol
 - 10 samples per matrix type
 - Advantage = MU « at large »
 - Heterogeneity of the sample contamination
 - Preparation of the initial suspension
 - Drawbacks
 - Need to repeat the protocol for each type of matrix analysed by the laboratory
 - Need to test naturally contaminated samples

Inter-laboratory s_R (method validation) (1)

- Advantages = available values
- Drawbacks
 1. Conditions to meet (see ISO/TS 21 748)
 - Laboratory's bias & precision
= compatible with the method's ones
 - All uncertainty sources (incl. test portion, sample preparation)
= taken into account in the inter-laboratory trial

Inter-laboratory s_R (method validation) (2)

- Drawbacks (foll.)
 2. Inter-laboratory s_R available for a limited number of methods in food microbiology
 3. Difficulty
 - to apply to routine analyses
 - precision data obtained in limited and artificial conditions
 - Combinaisons (matrix, strain)
 - Annex flora (if any)
 4. Risk to under-estimate MU
(samples homogenized and stabilized)

Inter-laboratory s_R (PT)

- Advantages
 - Available values
 - Large number of PT schemes in food microbiology
- Drawbacks
 1. Conditions to meet
 - Method used by the laboratory in PT = the same than in routine
 - Method used by a sufficient number of participants
 - PT samples \approx routine samples
 2. Samples

Measurement uncertainty in qualitative microbiology

- Preliminary works at ISO
- 2 possibilities foreseen
 - CI around the Limit of Detection (LoD_{50})
 - From the equivalent of reproducibility for qualitative determinations

Interpretation against legal limits

Legislative frame

- Food hygiene « package »
 - ↳ Regulation 852/2004 (« H I »)
- Draft Regulation on microbiological criteria (SANCO/4198 rev. 14, Dec. 2004)
- A microbiological criterion = a qualitative/quantitative limit +...

Food hygiene controls

- For operators' own checks
= MU not taken into account
 - For official food controls
= ?
 - the same rule
 - or (Figure 2)
 - o case (ii) for indicators
 - o case (iv) for pathogens
- To be precised into EC Guidelines, to come

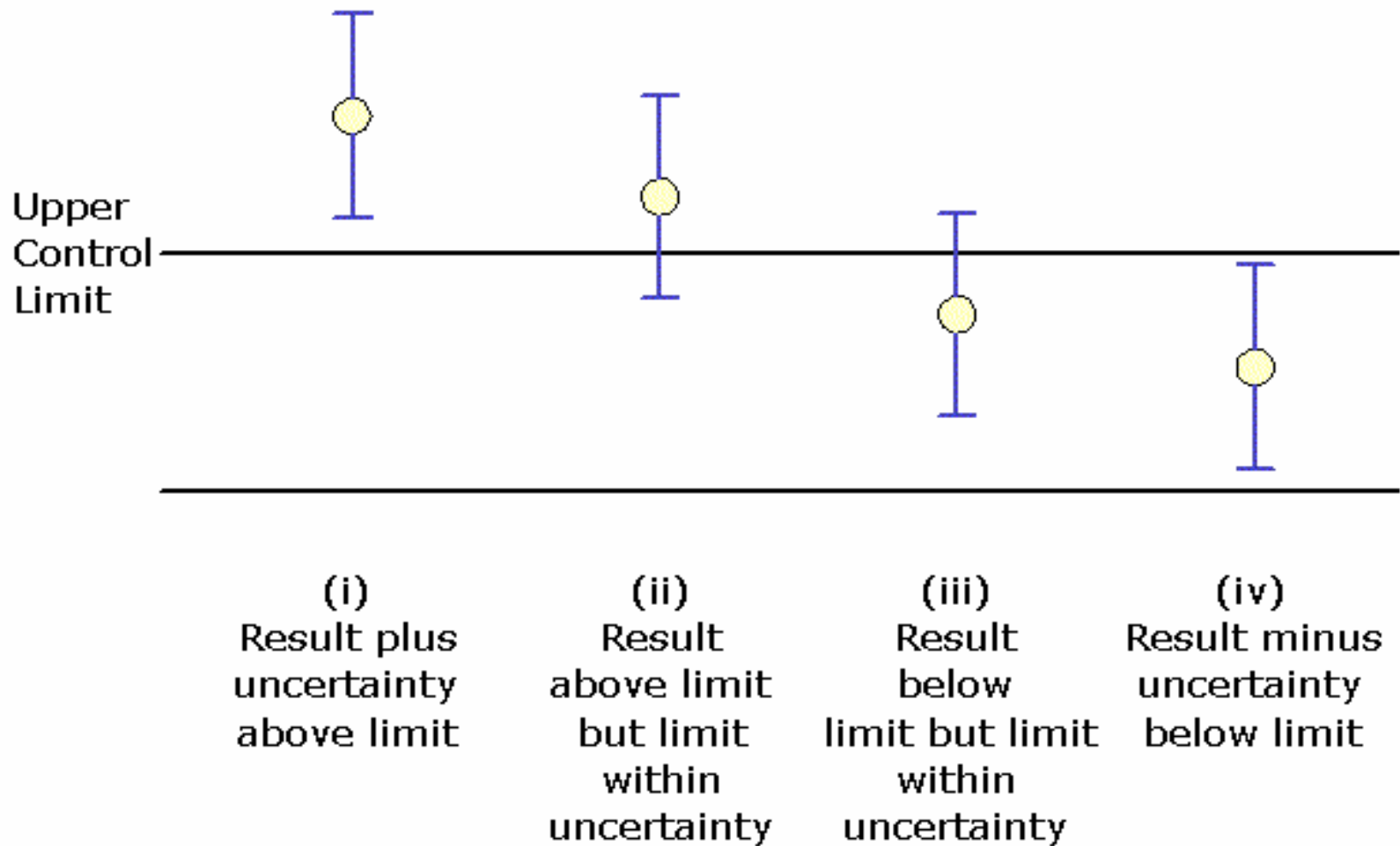


Figure 2: Uncertainty and compliance limits

Conclusion

- Global approach
 - Pragmatic, adapted to the complexity of
 - Food analysis
 - Microbiological analysis
 - In agreement with
 - GUM principles
 - International references and rules
- MU estimation becoming widespread in food microbiology
 - = Towards a more « scientific » analysis ?